REMARKS

Applicants respectfully requests entry of the amendments and remarks submitted herein. Claims 18-19, 32, 40, 42-43, 45-46, and 51 are amended; claims 1, 5, 8-10, 13, 21, 26-27, 29-30, 41, and 52 are canceled; and claim 53 is newly added. Therefore, claims 2-4, 6-7, 11-12, 14-20, 22-25, 28, 31-40, 42-51 and 53 are currently pending.

Support for the amendment to claim 19 is found in original claim 18.

Support for the amendment to claim 42 is found in original claim 32.

Support for the amendment to claim 18, 32, and 40 reciting a double-stranded oligonucloetide of at least about 20 bp but less than 200 bp, or is at least about 50 bp but less than about 200 bp, can be found throughout the specification, such as at page 26, lines 7-10.

Support for new claim 53 where the promoter is an RSV promoter can be found throughout the specification, such as at page 3, lines 6-7.

Rejections under 35 U.S.C. § 112, Second Paragraph

The Examiner rejected claim 43-50 under 37 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter.

(a) The Examiner rejected claim 43 (and claims 44-50 which depend from claim 43) concerning the term "weak promoter." The Examiner indicated that the metes and bounds of the weakness of the promoter required is not clear so that the skilled artisan would now whether a particular promoter would meet the limitation of the claim.

Applicant has amended claim 43 to remove the term "weak promoter." Applicant requests that this rejection be withdrawn.

(b) The Examiner rejected claim 45 concerning the term "the recombinase." Claim 45 has been amended to depend from claim 44, which recites "a recombinase," thereby providing adequate antecedent basis for "the recombinase" in claim 45. Applicant requests that this rejection be withdrawn.

(c) The Examiner rejected claim 46 concerning the term "the first and second site-specific recombination sequences." Claim 46 has been amended to depend from claim 44, which recites "first and second site-specific recombination sequences" thereby providing adequate

antecedent basis for "the first and second site-specific recombination sequences" in claim 46. Applicant requests that this rejection be withdrawn.

Rejections under 35 U.S.C. § 103(a)

A. Capecchi et al. in view of Sedivy et al.

The Examiner rejected claims 43-48 and 50 under 37 U.S.C. § 103(a), as being unpatentable over Capecchi et al. (U.S. Patent No. 5,631,153) in view of Sedivy et al. (Trends in Gen. 15:88-90 (1999).

Claim 43 recites a somatic cell gene targeting vector comprising a targeting vector comprising a gene targeting construct comprising a first cloning site operably linked to a DNA encoding a positive selection marker, a second cloning site and a first polyadenylation sequence, wherein the construct is promoterless, wherein the first cloning site comprises a first DNA segment that is homologous to a first genomic target sequence and the second cloning site comprises a second DNA segment that is homologous to a second genomic target sequence; and wherein the targeting vector comprises an expression cassette comprising a promoter operably linked to DNA encoding a negative selection marker and a second polyadenylation sequence, wherein the promoter is a Rous sarcoma virus (RSV) promoter, or a phosphoglycerate kinase (PGK) promoter. Claims 44-48 and 50 depend either directly or indirectly from claim 43.

The Supreme Court has set out the analysis for patentability under 35 U.S.C. § 103(a), which involves determining the scope and content of the prior art, ascertaining the differences between the prior art and the claims at issue, and resolving the level of ordinary skill in the pertinent art. Against this background the obviousness or nonobviousness of the subject matter is determined (*see, e.g., Graham v. John Deere Co.*, 383 U.S. 1 (1966) and *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727 (2007)). Further, the cited documents must be considered in their entirety, and it is not permissible to pick and choose from any one document only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such document fairly suggests to one of ordinary skill in the art (*see, e.g., Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve, Inc.*, 796 F.2d 443, 230 U.S.P.Q. 416 (Fed. Cir. 1986) and *In re Wesslau*, 353 F.2d 238, U.S.P.Q. 391 (C.C.P.A. 1965)). Applicants submit that the level of ordinary skill in the pertinent art is high. The scope and content of the prior art

and the differences between the prior art and the claims at issue are discussed hereinbelow, as are the reasons the claims are not obvious in view of any of the cited documents.

Capecchi et al. disclose positive-negative selector (PNS) vectors for modifying a target DNA sequence contained in the genome of a target cell capable of homologous recombination. The vectors comprise a first DNA sequence substantially homologous a first region of a target DNA sequence, a second DNA sequence substantially homologous to a second region of a target DNA sequence, a third DNA sequence positioned between the first and second DNA sequences that encodes a positive selection marker (which can be a promoterless positive selection marker), and a fourth DNA sequence encoding a negative selection marker. Capecchi et al. list a number of regulatory sequences for use with positive and/or negative selection markers in Table IIA and IIB.

Capecchi et al. do not disclose polyadenylation sequences operably linked to the positive selection marker, as recited by claim 43. Further, Capecchi et al. do not disclose excision of the positive selection sequences using site-specific recombination sequences, such as loxP sequences (as recited by claims 44-46). Instead, Capecchi et al. teach that the positive selection sequences can be excised by homologous recombination (col. 11, lines 3-6). They teach a detailed method where the positive selection marker is located in the intron of the targeted gene, and contains an independent functional promoter, *i.e.*, it is not promoterless (col. 11, lines 27-56). Moreover, Capecchi et al. do not teach or suggest either the RSV promoter or the PGK promoter.

Sedivy does not remedy the deficiencies of Capecchi et al. Sedivy discuss PNS vectors where the positively and negatively selectable genes are functionally independent expression cassettes, and each contains its own promoter and polyadenylation signals (p. 88, second column and Fig. 1). Sedivy does not teach a promoterless PNS vector, and therefore cannot teach the use of a polyadenylation sequence with a promoterless PNS vector, as recited in claim 1. Sedivy mentions theoretically the possibility of sequential targeting of second allele, but provides no technical details in this review. He cites a reference in which a cre/lox system was used in ES cells to recycle a targeting vector. In that paper, the cre/lox system is used to remove a PNS cassette (positive-negative selection cassette that includes both a promoter-driven neo resistance gene as well as a thymidine kinase gene). This might allow the PNS cassette to be used in subsequent rounds of targeting. In contrast, the present invention uses the site-specific recombination sequences to remove a promoterless neo gene (the promoterless neo gene is not

considered a PNS cassette). Since Sedivy does not teach or suggest a promoterless positive selection marker, Sedivy can not teach the combination of a promoterless positive selection marker in combination with a cre/lox system.

Therefore, since neither of the cited references teach nor suggest a promoterless PNS vector containing a polyadenylation sequence, the cited references even when combined do not teach or suggest all the features of claims 43-48 and 50. Further, since neither reference teaches or suggests the use of site-specific recombination sequences in a promoterless PNS vector, and since neither reference teach or suggest the use of a PGK or RSV promoter, claims 43-48 and 50 are not obvious over the cited art.

Applicant therefore requests the withdrawal of this rejection.

B. Capecchi et al. in view of Sedivy et al. and Barsoum

The Examiner has rejected claims 2-4, 6-7, 12, 14-18, 20, 22-25, 28, 31-37, 43-48 and 50-51 under 37 U.S.C. § 103(a), as being unpatentable over Capecchi et al. (U.S. Patent No. 5,631,153) in view of Sedivy et al. (Trends in Gen. 15:88-90 (1999) and further in view of Barsoum (U.S. Patent No. 4,956,288).

1. Claims 2-4, 6-7, 12, 14-18, 20, 22-25, 28, 31-37, and 51

Independent claim 18 recites a method for disrupting a gene of interest in a somatic cell *in vitro*, which method comprises introducing a targeting vector comprising a gene targeting construct comprising a first cloning site operably linked to a DNA encoding a positive selection marker, a second cloning site and a first polyadenylation sequence, wherein the construct is promoterless; and an expression cassette comprising a promoter operably linked to DNA encoding a negative selection marker and a second polyadenylation sequence, wherein the first cloning site comprises a first DNA segment that is homologous to a first genomic target sequence and the second cloning site comprises a second DNA segment that is homologous to a second genomic target sequence, into a somatic cell such that the first genomic target sequence and the second genomic target sequence recombine with the gene to yield a genetically altered cell, further comprising introducing a double-stranded oligonucleotide of at least about 20 bp but less than about 200 bp into the somatic cell. Claims 2-4, 6-7, 12, 14-17, 20, 22-25, 28, 31, and 51 depend either directly or indirectly from claim 18.

Independent claim 32 recites a somatic cell gene targeting transfection mixture comprising a gene targeting construct comprising a first cloning site operably linked to a DNA encoding a positive selection marker, a second cloning site and a first polyadenylation sequence, wherein the construct is promoterless, wherein the first cloning site comprises a first DNA segment that is homologous to a first genomic target sequence and the second cloning site comprises a second DNA segment that is homologous to a second genomic target sequence; and an expression cassette comprising a promoter operably linked to DNA encoding a negative selection marker and a second polyadenylation sequence, and a double-stranded oligonucleotide of at least about 20 bp but less than about 200 bp. Claims 33-37 depend either directly or indirectly from claim 32.

Neither Capecchi et al. nor Sedivy et al. teach a method comprising introducing a double-stranded oligonucleotide into the somatic cell along with the targeting vector. Barsoum does not remedy the deficiencies of Capecchi et al. and Sedivy et al. Barsoum teaches the inclusion of carrier DNA in a transformation mixture. In particular, Barsoum teaches that the carrier DNA is approximately 300 to 1000 bp in length (col. 10, lines 52-53). Barsoum, however, does not teach or suggest the inclusion of a double-stranded oligonucleotide of at least about 20 bp but less than about 200 bp as recited by claims 2-4, 6-7, 12, 14-18, 20, 22-25, 28, 31-37 and 51.

Since even when combined the references do not teach or suggest all the features of pending claims 2-4, 6-7, 12, 14-18, 20, 22-25, 28, 31-37 and 51, the pending claims are not obvious over the cited references. Applicant, therefore, requests that this rejection be withdrawn.

2. Claim 43-48, and 50

Independent claim 43 recites a somatic cell gene targeting vector comprising a targeting vector comprising a gene targeting construct comprising a first cloning site operably linked to a DNA encoding a positive selection marker, a second cloning site and a first polyadenylation sequence, wherein the construct is promoterless, wherein the first cloning site comprises a first DNA segment that is homologous to a first genomic target sequence and the second cloning site comprises a second DNA segment that is homologous to a second genomic target sequence; and wherein the targeting vector comprises an expression cassette comprising a promoter operably linked to DNA encoding a negative selection marker and a second polyadenylation sequence, wherein the promoter is a Rous sarcoma virus (RSV) promoter, or a phosphoglycerate kinase (PGK) promoter. Claims 44-48 and 50 depend either directly or indirectly from claim 43.

As discussed above, neither Capecchi et al. nor Sedivy et al. teach nor suggest the use of a promoterless PNS vector containing a polyadenylation sequence, the use of site-specific recombination sequences in a promoterless PNS vector, or the use of a PGK or RSV promoter. Barsoum does not remedy the deficiencies of Capecchi et al. and Sedivy et al. Barsoum teaches the inclusion of carrier DNA in a transformation mixture. Barsoum, however, does not teach or suggest the use of a PGK or RSV promoter.

Since even when combined the references do not teach or suggest all the features of pending claims 44-48 and 50, the pending claims are not obvious over the cited references. Applicant, therefore, requests that this rejection be withdrawn.

C. Capecchi et al. in view of Sedivy et al., Barsoum and Pfarr et al.

The Examiner has also rejected claim 49 under 37 U.S.C. § 103(a), as being unpatentable over Capecchi et al. (U.S. Patent No. 5,631,153) in view of Sedivy et al. (Trends in Gen. 15:88-90 (1999) further in view of Pfarr et al. (DNA, 1986, Vol. 5, No. 2, pages 11-122).

Claim 49 recites the somatic cell gene targeting vector of claim 43, wherein the expression cassette comprises a BGH polyadenylation sequence.

As discussed above, neither Capecchi et al. nor Sedivy et al. teach nor suggest the use of a promoterless PNS vector containing a polyadenylation sequence, the use of site-specific recombination sequences in a promoterless PNS vector, or the use of a PGK or RSV promoter. Pfarr et al. does not remedy the deficiencies of Capecchi et al. and Sedivy et al. Pfarr et al. teaches a comparison of various polyadenylation regions on gene expression in mammalian cells using a downstream galactokinase marker gene. Pfarr et al., however, does not teach or suggest the use of a PGK or RSV promoter.

Since even when combined the references do not teach or suggest all the features of pending claim 49, the claim 49 is not obvious over the cited references. Applicant, therefore, requests that this rejection be withdrawn.

D. Capecchi et al. in view of Sedivy et al., Barsoum and Pfarr et al.

The Examiner has also rejected claims 11 and 38 under 37 U.S.C. § 103(a), as being unpatentable over Capecchi et al. (U.S. Patent No. 5,631,153) in view of Sedivy et al. (Trends in

Gen. 15:88-90 (1999), in view of Barsoum (U.S. Patent No. 4,956,288) and in view of Pfarr et al. (DNA, 1986, Vol. 5, No. 2, pages 11-122).

Claim 11 recites the method of claim 18 (*i.e.*, a method for disrupting a gene of interest in a somatic cell *in vitro*, which method comprises introducing a targeting vector comprising a gene targeting construct comprising a first cloning site operably linked to a DNA encoding a positive selection marker, a second cloning site and a first polyadenylation sequence, wherein the construct is promoterless; and an expression cassette comprising a promoter operably linked to DNA encoding a negative selection marker and a second polyadenylation sequence, wherein the first cloning site comprises a first DNA segment that is homologous to a first genomic target sequence and the second cloning site comprises a second DNA segment that is homologous to a second genomic target sequence, into a somatic cell such that the first genomic target sequence and the second genomic target sequence recombine with the gene to yield a genetically altered cell, further comprising introducing a double-stranded oligonucleotide of at least about 20 bp but less than about 200 bp into the somatic cell), wherein the second polyadenylation sequence comprises a BGH polyadenylation sequence.

Claim 38 recites the somatic cell gene targeting transfection mixture of claim 32 (i.e., a somatic cell gene targeting transfection mixture comprising: a gene targeting construct comprising a first cloning site operably linked to a DNA encoding a positive selection marker, a second cloning site and a first polyadenylation sequence, wherein the construct is promoterless, wherein the first cloning site comprises a first DNA segment that is homologous to a first genomic target sequence and the second cloning site comprises a second DNA segment that is homologous to a second genomic target sequence; and an expression cassette comprising a promoter operably linked to DNA encoding a negative selection marker and a second polyadenylation sequence, and a double-stranded oligonucleotide of at least about 20 bp but less than about 200 bp), wherein the expression cassette comprises a BGH polyadenylation sequence.

As discussed above neither Capecchi et al. nor Sedivy et al. teach a method comprising introducing a double-stranded oligonucleotide into the somatic cell along with the targeting vector. Barsoum does not remedy the deficiencies of Capecchi et al. and Sedivy et al. Barsoum teaches the inclusion of carrier DNA in a transformation mixture. In particular, Barsoum teaches that the carrier DNA is approximately 300 to 1000 bp in length (col. 10, lines 52-53). Barsoum,

however, does not teach or suggest the inclusion of a double-stranded oligonucleotide of at least about 20 bp but less than about 200 bp as recited by the pending claims.

Pfarr et al. does not remedy the deficiencies of Capecchi et al., Sedivy et al., and Barsoum. Pfarr et al. teaches a comparison of various polyadenylation regions on gene expression in mammalian cells. Pfarr et al. do not teach or suggest the inclusion of a double-stranded oligonucleotide of at least about 20 bp but less than about 200 bp as recited by the pending claims. Applicant, therefore, requests that this rejection be withdrawn.

Allowable Subject Matter

The Examiner has indicated that claims 19 and 42 would be allowable if re-written in an independent form. Claims 19 and 42 have been re-written as suggested by the Examiner.

CONCLUSION

The Examiner is invited to contact Applicant's Representative at the below-listed telephone number if there are any questions regarding this Response or if prosecution of this application may be assisted thereby. If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 50-3503. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extension fees to Deposit Account 50-3503.

Respectfully submitted,

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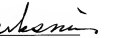
By: 
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